CONTROL ID: 2531274

TITLE: CFTR Potentiator PG-01 and Corrector KM-11060 can rescue hERG mutations trafficking

PRESENTATION TYPE: General Communication

CURRENT SPECIAL INTEREST GROUP: Cellular Signalling.

Theme GC: Epithelia & Membrane Transport

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ABSTRACT BODY:

Abstract Body : Type II congenitalLong QT syndrome (LQT2) is due to genetic mutations in hERG channel. Genetic or pharmacological factors could potentially affect hERG channel biogenesis and contributes to LQTS, for example, disease mutations G601S and T473P result in hERG trafficking deficiency [1,2]. Various rescue strategies for hERG dysfuction are being developed. Some correctors for CFTR channel have been reported to act indirectly on proteostasis pathways to promote folding and correction on hERG trafficking deficiency [3]. In this study, we tested the hypothesis that the CFTR corrector KM-11060 and the potentiator PG-01 may correct hERG mutation trafficking diseases.

We use HEK293 cell line expressing a well-studied trafficking disease mutation G601S-hERG channel [4]. We treated cells with CFTR potentiator PG-01and corrector KM-11060, which function through different cellular mechanisms, and assessed whether correction occurred via immunoblotting. Whole cell proteins from HEK 293 cells expressing hERG channels were used for analysis [5]. Proteins were separated on 8% SDS-polyacrylamide electrophoresis gels for 1 hour, transferred onto PVDF membrane, and blocked for 1 h with 5% nonfat milk. The blots were incubated with the primary antibody (Santa Cruz Biotechnology) for 12-16 h at 4C temperature and then incubated with a donkey anti-goat horseradish peroxidase-conjugated secondary antibody(Santa Cruz Biotechnology). Actin expression was used for loading controls. The blots were visualized using the ECL detection kit (Genshare).Results were deemed significantly different from controls by a one-way ANOVA ($p < 0.05$).

Our results show that both KM-11060 (5, 10, 20uM) and PG-01(5, 15 uM) can correct G601S mutant alleles of hERG protein trafficking (Fig 1, 2). KM-11060 (20uM) but not PG-01(15 uM) enhance protein expression of wild type hERG channel (Fig 2). Further treatment on cells at low temperature with different drug concentration will be tested. Functional studies are also needed to test whether the drugs can correct the function of hERG mutation channel. These results could potentially provide novel insight into the correction mechanism of CFTR potentiator and also help to develop new treatment for LQT2.

Reference 1: Smith JL, et al. Am J Physiol Cell Physiol, 201,301:C75-85

Reference 2: Liu L, et al. Heart Rhythm, 2013,10:61-67

Reference 3: Sampson HM, et al. Orphanet J Rare Dis. 2013, 8:11

Reference 4: Li G et al. Mol Med Rep. 2016, 13(3):2467-75

Reference 5: Guo J, et al. J Biol Chem. 2011, 286(40):34664-74

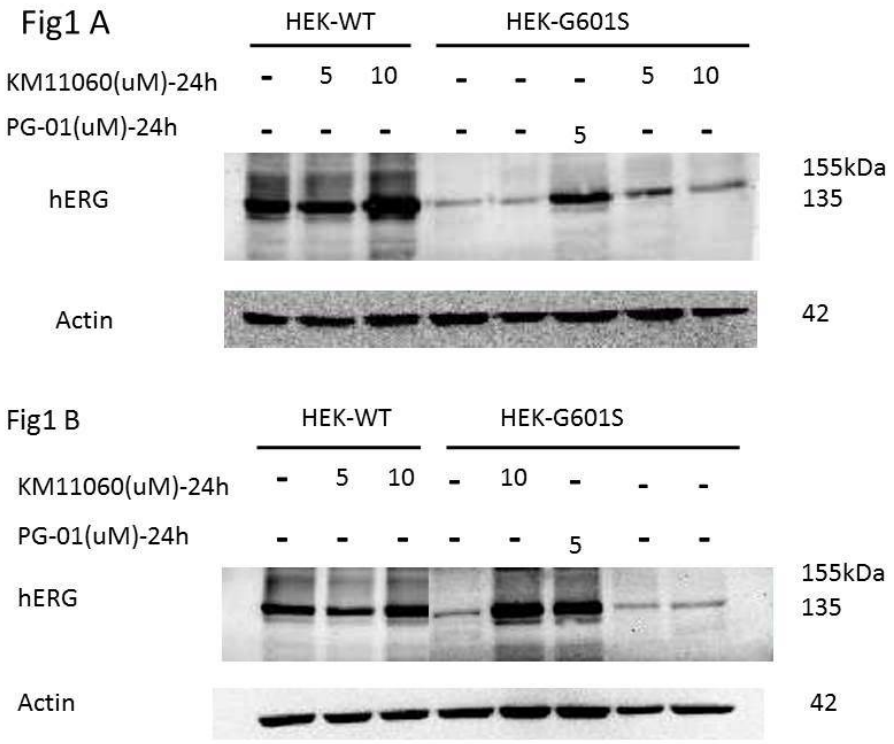

Fig1. Representative blot showing WT-hERG or G601S-hERG vector transiently transfected HEK293 cell line before and after KM11060(5,10uM) or PG-01(5uM) treatment. 135kDa and 155kDa indicate core and complex-glycosylated hERG separately. Actin serves as a loading control;(A) WT-hERG express both core and complex-glycosylated protein, while G601S-hERG only express 135kDa; KM11060(5,10uM) has no influence on WT-hERG protein expression, while they and PG-01(5uM) can partly correct G601S-hERG which showing 155kDa protein after the treatment;(B) the same with (A).

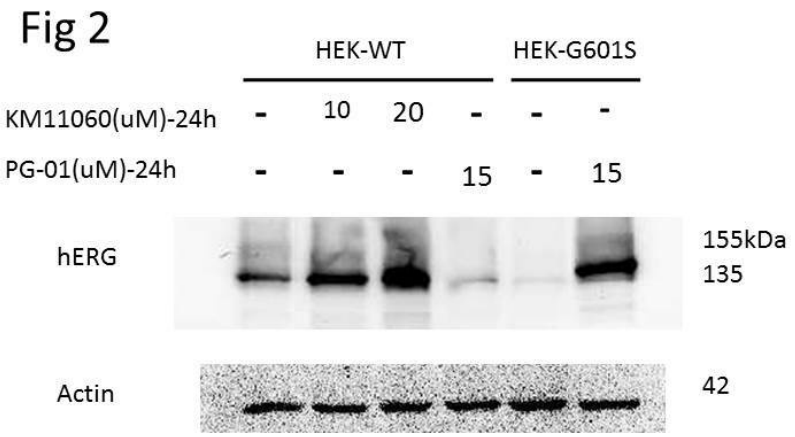

Fig2. Representative blot showing WT-hERG or G601S-hERG vector transiently transfected HEK293 cell line before and after KM11060 (10,20uM) or PG-01(15uM) treatment. 135kDa and 155kDa indicate core and complex-glycosylated hERG separately. KM11060 (10uM) has no influence on WT-hERG expression, but 20uM has increased the expression of WT-hERG; PG-01(15uM) has not affect WT-hERG expression, while the same dosage can partly correct G601S-hERG which present 155kDa after the treatment.

IMAGE CAPTION: Fig1. Representative blot showing WT-hERG or G601S-hERG vector transiently transfected HEK293 cell line before and after KM11060(5,10uM) or PG-01(5uM) treatment. 135kDa and 155kDa indicate core and complex-glycosylated hERG separately. Actin serves as a loading control;(A) WT-hERG express both core and complex-glycosylated protein, while G601S-hERG only express 135kDa; KM11060(5,10uM) has no influence on WT-hERG protein expression, while they and PG-01(5uM) can partly correct G601S-hERG which showing 155kDa protein after the treatment;(B) the same with (A). Fig2. Representative blot showing WT-hERG or G601S-hERG vector transiently transfected HEK293 cell line before and after KM11060 (10,20uM) or PG-01(15uM) treatment. 135kDa and 155kDa indicate core and complex-glycosylated hERG separately. KM11060 (10uM) has no influence on WT-hERG expression, but 20uM has increased the expression of WT-hERG; PG-01(15uM) has not affect WT-hERG expression, while the same dosage can partly correct G601S-hERG which present 155kDa after the treatment.

TABLE TITLE: (No Tables)

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